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MEDITERRANEAN AND BLACK SEA MACROFAUNA: COMPARING SAMPLING STRATEGIES

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Aplicada, realizada sob a orientação científica do Professor Victor Quintino, Professor Auxiliar do Departamento de Biologia da Universidade de Aveiro, e co-orientaçao da Professor Alberto Basset, Full Professor do Department of Biological and Environmental Sciences and Tecnologies of the University of Salento (Italy).

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palavras-chave

Macrofauna bentónica, índices bióticos, águas de transição, corer, sacos de folhas, salinidade

resumo

A implementação da Directiva Quadro da Água tem levantado importantes questões relativas aos aspectos metodológicos da amostragem das comunidades bentónicas, tendo sido feitas várias tentativas no sentido de estandardizar as estratégias de amostragem, especialmente em águas de transição.

Este estudo pretende abordar esta questão através da comparação das comunidades de macrofauna amostradas com dois métodos distintos, corer e sacos de folhas. O trabalho foi realizado no Mediterrâneo e no Mar Negro, em 10 ecossistemas de transição, distintos, sobretudo, ao nível da salinidade e tipologia de habitat.

No conjunto, foram amostrados 172 taxa dos quais, 49 apenas com corers e 52 apenas com sacos de folhas. Setenta e um taxa foram comuns a ambos os amostradores. Os artrópodes são comuns aos dois amostradores, sendo dominantes nos sacos de folhas, enquanto os anelídeos caracterizam os corers.

A riqueza taxonómica média por amostra foi de 4,5 nos corers e 5,2 nos sacos de folhas. Quer os corers quer os sacos de folhas apresentaram padrões semelhantes no que diz respeito às principais características estruturais da comunidade. Dos sistemas oligohalinos (<5 ‰) para os sistemas euhalinos (>30 ‰), a diversidade taxonómica (H') e a similitude de Bray-Curtis mostraram o mesmo padrão de variação. Apesar destas semelhanças, as comunidades de macrofauna bentónica amostradas com os dois dispositivos mostraram diferenças significativas que não foram relacionadas, ao nível da escala eco-regional, com o gradiente salino ou a tipologia do habitat.

As diferenças entre os amostradores mantiveram-se significativas quando a análise foi realizada com base em índices bióticos, entre os quais alguns índices de qualidade ecológica. Os resultados obtidos no presente trabalho têm implicações na avaliação e monitorização do estado ecológico dos sistemas de transição, pondo em evidência o facto dos corers e dos sacos de folhas recolherem amostras caracterizadas por diferentes espécies. No entanto, a diferenças entre as lagoas mostrou-se mais importante do que a diferença entre ecótipos, isto é, grupos de salinidade.

keywords Benthic macrofauna, biotic indices, transitional waters, corer, leaf bag, salinity.

abstract

One of the important questions in the implementation of the Water Framework Directive regards the methodological aspects about the sampling of benthic communities and many attempts are made in order to standardize sampling strategies, especially in transitional waters.

The present study aims to compare the macrofauna communities colonizing leaf bags and those obtained from box-corer samples. The study was carried out in 10 transitional aquatic ecosystems located in the Mediterranean ,the Black Sea and differing mainly in water salinity and habitat typology.

Overall, a total of 172 taxa were sampled; 49 taxa were found only in the sediment samples and 52 only in the bags; 71 were common to both samplers. Arthropods were a common component to both samplers, dominant in leaf bags, while annelids characterized mainly the corers.

Mean taxonomic richness per sample was 4,5 in the corers and 5,2 in leaf-bags. Both corer and leaf-bag samples showed similar patterns of the main community structural characteristics. From the oligohaline (<5‰) to the euhaline (>30‰) systems, taxonomic diversity (H') and Bray-Curtis similarity showed consistent patterns of variation. Despite these similarities, The benthic macrofauna sampled with the two devices showed significant differences that were not related, on ecoregional scale, to the salinity gradient or the habitat typology.

The differences between samplers remained significant also when the analysis was performed on biotic indices, namely some ecosystem quality indices. The results obtained in the present work have major implication for the assessment and monitoring of ecological status in transitional waters. They evidence that the benthic invertebrates upon which the taxonomic indices are calculated and those that contribute to the functional aspects based on the study of decomposition rates, are essentially distinct. However, on the studied spatial scale, the lagoon heterogeneity (differences among lagoons) were more important than the ecotopes (i.e., salinity groups).

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1. Introduction

1.1. General subject

The utility of benthic macroinvertebrates for assessing environmental conditions in aquatic ecosystems has long been recognized (Cairns and Pratt, 1993), and more recently invertebrates have received appreciable attention on impact assessment and qualitative classification of transitional aquatic systems (Weisberg et al., 1997; Magni et al., 2009). One of the major advantages of biomonitoring with benthic macroinvertebrates is the possibility to detect changes in water quality that occur at the time of sampling as well as changes that have occurred within a longer period before sampling, due to the relatively sedentary life style and long life spans of these organisms (Rosenberg and Resh, 1996; Schwoerbel, 1999). So they can integrate changes in the physical and chemical environment over time and space, are key contributors to chemical fluxes over the sediment-water interface, and present well-established response models to anthropogenic pressures (Cook, 1976; Pearson and Rosenberg, 1978; Aller and Aller, 1998).

In comparison, chemical and physical analysis might be more accurate, but these only reflect the actual conditions in the water body at the time of sampling (Alba-Tercedor, 1996). Furthermore, macroinvertebrates show sensitivity towards various factors that are responsible for changes in water quality, and they are less expensive to work with than chemical and physical analysis (Pontasch and Cairns, 1991).

Many bio-monitoring programs in the aquatic environment rely on the analysis of communities' structural aspects and more recently macrobenthic communities have also been included among the biological elements indicated in the European Water Framework Directive, passed by the EU Parliament in June 2000 (WFD, 2000/60/EC). The Directive provides a framework for conserving and protecting the ecological integrity of the most significant water bodies, including transitional waters and has established the framework, leaving the precise method for determining the ecological status to be defined. Hence, there is the necessity, as well as the opportunity, to set up a standardized assessment method for aquatic environments and to define unambiguous quality targets for waters throughout

Europe. Furthemore, the European Water Framework Directive has instituted the concept of Ecological Quality Statement (EQS) as a way to assess the biological quality of waters. The EQS is based mainly upon the composition of benthos and other biological compartments in the ecosystem with respect to reference sites (Dauvin and Ruellet, 2007). Such tools, useful to ecosystem management, are already well established and standardized for freshwaters, but not for coastal and transitional waters. Although in recent years, several biotic indices, already defined for marine environments, have been proposed to be used as ecological indicators in transitional aquatic ecosystems (e.g., AMBI [Borja et al., 2000] BENTIX [Simboura and Zenetos, 2002], BQI [Rosenberg et al., 2004] and BOPA [Dauvin and Ruellet, 2007]), many uncertainties still persist and further studies are required to standardize the existing indices in these types of ecosystems.

1.2. Transitional aquatic ecosystems

Transitional aquatic ecosystems or more simply ‘transitional waters’ are open systems, ecotones between land, sea and fresh waters, which are continuously modified by the materials they receive from land, sea and fresh waters. Their origin as ecotones determines an internal heterogeneity, which can be represented by salinity and nutrient boundaries or energy variation thresholds (Legendre and Demers, 1985), as well as a heterogeneity among ecosystems, which can be characterized by very different terrestrial-freshwater, freshwater-marine interfaces. They represent important and fragile ecosystems in the coastal Mediterranean landscape, providing key ecosystem services, such as water quality improvement, by reducing the pollution loads transported by the rivers in coastal water environments, fisheries resources and recreational areas for human populations, and habitat and food for migratory and resident animals (Levin, 2001). The balance of water, salt, nutrients, particulate organic and inorganic matter sets spatial boundaries within transitional ecosystems, which are described according to the Confinement (Guelorget and Pertuisot, 1983) and Ergocline (Legendre and Demers, 1985) theories. Variations of these balances with time may also set temporal boundaries discriminating ecological conditions, or environmental niches (sensu Emlen, 1973), which support strikingly different community structures. The response time of transitional water communities to the changing environment, determining the time scale of ecological successions, is a critical factor in transitional water ecosystems affecting the likelihood of alternative equilibrium states or ‘transitional states’. Shifts between not-confined and confined ecosystems, freshwater and marine guilds, seagrass, seaweed and phytoplankton dominated producer guilds, represent some examples. These recognizable community structure units, which may potentially evolve one into another as a function of the water, salt, nutrient, particulate organic and inorganic matter balances, can be defined as transitional states of transitional waters. As structure and functioning of transitional aquatic ecosystems are threatened by a number of human activities involving water flow alteration, eutrophication and chemical pollution, over-exploitation and land reclamation, transition from one state to another is often triggered by a combination of natural (e.g. temperature changes, freshwater flooding, etc.) and human (e.g. nutrient loading, toxic chemicals, habitat fragmentation, etc.) derived stresses. The inclusion of transitional waters in all biomonitoring programs would be necessary due to their ecological importance and fragility.

1.3. Transitional waters and biomonitoring

According to the Water Framework Directive (WFD) definition, Transitional Waters (TWs) are ‘bodies of surface water in the vicinity of river mouths which are partially saline in character as a result of their proximity to coastal waters but which are substantially influenced by freshwater flows’ (European Communities, 2000). They are characterized by highly dynamic physical, chemical and hydro-morphologic conditions, resulting in a mosaic of habitats in which species are particularly well adapted to variability. Such species are essentially more tolerant to change than their marine counterparts, which translates into a scientific challenge to develop suitable quality indicators for transitional waters (Elliott and Quintino, 2007). Due to their specific characteristics, the EU WFD posed to the scientific community the challenge to classify these ecosystems into a small number of types as important requirement for their inclusion in the monitoring programs.

As in the most aquatic ecosystems, in transitional waters, data on benthic aquatic invertebrates have been obtained through works aiming at investigate structural ecosystem characteristics or functional processes. Biomonitoring programs to access the ecological integrity, mainly of freshwaters, tend to rely exclusively on structural parameters using benthic macroinvertebrate derived metrics and biotic indices as measures of structural integrity. Given that, anthropogenic stress can induce changes also in ecosystem processes (Gessner et al., 2004), the use of functional assessment methods, such as litter breakdown, have been used more recently as tool to assess ecosystem integrity (Gessner and Chauvet, 2002). Moreover, several studies include both structural and functional approaches to ecosystem biomonitoring, and compare litter breakdown rates with structural characteristics of the associated benthic macrofauna; these studies are recent and have been carried mainly in freshwater ecosystems (Nelson, 2000; Lecerf et al., 2006, Castela et al., 2008, Mckie and Malmqvist, 2009). On the other hand, very few studies (Quintino et al., 2011) are available for coastal or estuarine environments, aimed at comparing taxonomic composition and structure of benthic communities obtained through traditional methods, as corer or grab, and through litter bag samples, as those used in decomposition studies.

2. Objectives and experimental design

The WFD of the European Union (EC, 2000) necessitates the use of benthic invertebrates in the assessment and monitoring of aquatic environments. So the comparison of different sampling methods of benthic macrofauna is an important question in order to implement the WFD about the methodologies used for sampling benthic communities, with reference mainly to transitional waters. Moreover, not only one of the important aspects of the implementation of WFD regards these methodological aspects, but also we have very few information about how close are the communities on which rely the taxonomic based indices and those which contribute to the functional indicators. In the present work such issue is addressed through the comparison of macroinvertebrate communities sampled by two techniques, a box-corer to obtain quantitative sediment samples and litter bags of *Phragmites australis* leaves placed at the sediment surface. Moreover, the study is aimed also at investigate potential differences between macrofauna sampled through the two devices, in relation to some sources of lagoon heterogeneity selected on the basis of the Mediterranean and Black Sea ecosystem typologies set out in Basset et al. (2008).

A nested experimental design was performed, with samples obtained in ten ecosystems, in a different number of station depending on the surface area of each ecosystem and five replicates per sampling station. Litter bags with 5 mm mesh size were used in the study of litter breakdown in the same ecosystems (Sangiorgio et al., 2008). The benthic communities obtained by the two approaches were compared in a model using the sampling devices and water salinity/habitat typologies as fixed and crossed main factors, the lagoons nested in salinity (or habitat typologies) and the sampling sites nested in lagoons, under the null hypothesis that no significant differences would exist between the main factors and their interaction term.

3. Materials and methods

3.1. *Study sites*

Data presented in this study are a part of an European Project; specifically this work was based on data collected in 10 transitional aquatic ecosystems of the Central, Adriatic, Danubian and South-eastern European Space (CADSES area), including three countries (from west to east: Italy, Albania and Romania). Data were collected during the TWReferenceNET Project, as part of the Interreg IIIB CADSES (Central European, Adriatic, Danubian, South-Eastern, European Space) Program 140 (Figure 1). The group of studied ecosystems was selected in order to represent heterogeneity of Mediterranean and Black Sea lagoons. The ecosystems included micro- and non-tidal lagoons (following Basset et al., 2006), salt pans and almost freshwater coastal wetlands; two ecosystems, Grado Cavanata and Grado fish farm, are actually compartments of the large Grado-Marano lagoon complex. The selected ecosystems differed mainly in water salinity, but also in habitat typology; however the ecosystems were also classified as disturbed and undisturbed (Barbone et al., 2012; Basset et al, 2012).

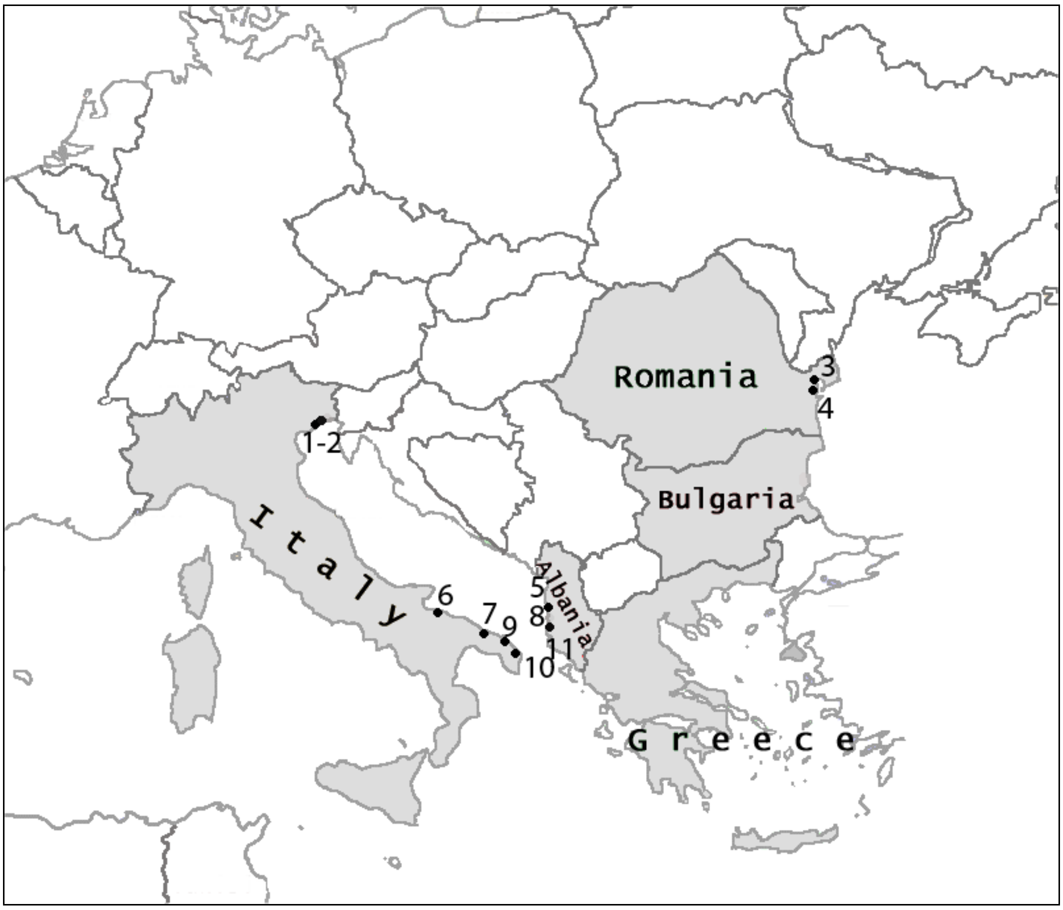


Figure 1. Geographic localization of the ten studied transitional water ecosystems within the CADSES area (in grey). 1=Grado fish farm, 2=Grado Cavanata, 3=Leahova, 4=Sinoe, 5=Patok, 6=Margherita di Savoia, 7=Torre Guaceto, 8=Narta, 9=Cesine, 10=Alimini.

As regards Italy, the work was carried out in different lagoons from North to South: Grado fish farm, Grado Cavanata, Margherita di Savoia, Torre Guaceto, Cesine and Alimini.

The Cavanata Valley includes two basins with 0.1 km^2 and 0.21 km^2, respectively. In the valley there is aquaculture with the fishes entering to the valley when the drains are opened. Fish farm is an embanked lagoon area, mean depth of 80 cm, where the aquaculture activities are carried out.

Margherita di Savoia salt pans are located along the south-eastern coast of the Adriatic Sea. The total surface area is about 40 Km^2 with a mean depth of 2.5 m. They are connected to the Adriatic Sea by a channel 2350 m long and about 4 m deep. The salt pans consist of two different basins linked to each other: the first has a surface area of 35 Km^2 and is used for the process of evaporation, while the second, with a surface area of 5 Km^2 , is used for the process of production of salt.

The Nature Reserve of Torre Guaceto is located in Apulia, along the Adriatic Sea coast, at about 15 Km North of Brindisi. The area is characterized by a Mediterranean climate, with hot and dry spring-summer period, stable from a meteorological point of view, and a cold and wet autumn-winter period, more changeable. The complex of the aquatic systems is composed by the wet brackish area, present in the terrestrial reserve and partitioned with a seasonal trend, and by the sea area facing the reserve. The brackish area extent is of about 1.20 km^2. The wet area is crossed by a network of canals, made in the past in order to reclaim the marshy area, and that bound several areas with changeable extent; the bed of reeds is half crossed by a rubble road, that is submerged during the autumn-winter season while, during the dry season, it separates the brackish ecosystem in two distinct sections.

Cesine lagoon is a shallow coastal water body in South East Italy. The saltmarsh area of Cesine includes several temporary and permanent ponds among which Pantano Grande is the largest. Pantano Grande covers an area of 0.68 km^2 and has an average depth of 0.80 m. Pantano Grande is separated from the Adriatic Sea by coastal dunes that prevent water from returning to the sea and receives freshwater from rainfall.

Alimini is a salt-marsh ecosystem on the Adriatic coast of southern Italy, in the Salento peninsula. It covers an area of 1.37 km^2 and measures 2.86 km by 1.54 km. The total length of the shoreline is 9.53 km; it has an irregular elongated shape (sinuosity index: 2.29) with the major axis parallel to the coast and it is completely exposed to the dominant winds. The mean depth of the lake is 1.50 m, but the water level varies depending on marine and freshwater inputs. The lake is connected to the sea through its mouth and with a freshwater lake, Alimini Piccolo, through a natural canal 1.5 km long, called the “Strittu”. The lake receives three main freshwater inputs: one from Alimini Piccolo in the South, a second from a small stream (Zuddeo) in the North-West and a second from three small canals entering the north side of the lake, carrying water from the Traugnano Swamp; moreover, it receives seawater input from the Adriatic Sea to the East.

As regards Albania, the work was carried out in the lagoons of Narta and Patok. Narta is situated in the Vlora region. It is one of the largest and most important lagoons in Albania with an area of 42 km^2, from which 13.8 km^2 are used for salt production in the salt mine of Skrofotina. The lagoon depth is from 0.70 m to 1.50 m. The salt mine in Narta is one of the oldest in the Balkans and Albania. Salt production till 1990 was 140-150 thousand tones per year.

The lagoonary complex of Patok represents one of the most interesting natural beauties of that Albanian coast; situated between River Mati in the North and River Ishmi in the South, this complex includes a high diversity of habitats: internal lagoon and external lagoon (4.8 km^2). The favourable position and diversity of habitats around the lagoon have created suitable conditions for a rich fauna of birds.

As regards Romania, the study was carried out in the transnational region of the Danube delta. Particularly the study was dealt with the transitional ecosystems (Leahova and Sinoe) inside the protected areas occurring in the complex of Lakes Rosu – Puiu – Caraorman, which is one of the largest natural areas in the Danube Delta that includes a high variety of biodiversity. The total surface of the complex is about 450 km^2 while the surface of the reserve is of 5.8 km^2. In this complex there is a high variety of habitats and ecosystems including different types of wetlands (running water, lakes, marshes), forests, reed beds, floating reed-bed islands, grassland. These ecosystems ensure habitats for a large number of plants and animals including colonies of pelicans and several species of freshwater fish. The fish populations, reed-beds and grasslands represent important financial sources for the about 4000 people living in the villages close to the complex. In the final decades of last century, especially between 70s and 90s, the economic activity developed by the communist regime affected the equilibrium of the natural ecosystems. An important plant for exploitation the sand was placed near the Caraorman Forest, one of the 18th Strictly Protected Areas in the Danube Delta Biosphere Reserve.

3.2. *Field and laboratory procedures*

Field sampling campaigns were carried out in two sampling times during the year (autumn and spring) for box-corer samples and only in spring for leaf bag samples. In this study we refer to the data obtained in spring. Sampling sites in each ecosystem were chosen so as to include a variety of habitats (mud/sand, with/without submerged and emerged vegetation).

Sediment samples were collected by standard benthic method using a Reineck box-corer (0.03 m^2); for each sampling site, five replicates were collected. Each sample was sieved through a 0.5 mm mesh sieve and the material retained in the sieve was fixed in 4% buffered formalin. In the laboratory, all samples were individually hand-sorted and the benthic macrofauna were selected under a stereomicroscope. All animals were later identified to species level whenever possible, using binocular stereoscopic and optical microscopes. Each individual was weighed to the nearest 1 mg after drying for 72 h at 60°C. Ash content was obtained at the individual level (for large species) or on groups of con-specific individuals (for small species) after muffle furnace combustion for 6 h at 500°C. All data regarding individual body sizes are then expressed as individual biomass (AFDM) after calculation of the individual ash-free dry weight (AFDW). Species were assigned to feeding guilds (collector-filterers, predators, shredders/scrapers) on the basis of literature records.

An experimental field study of detritus decomposition was undertaken in the same stations on leaves of *Phragmites australis*, using the litter bag technique (Bocock and Gilbert, 1957; Shanks and Olson, 1961). Leaves were collected at the same time and from the same area at the beginning of autumn; the basal and apical parts of all leaves were cut off, and only the central leaf section was used. In spring, litter bags (0.5 cm mesh size) were filled with 3.000+0.005 g of oven-dried leaves (60 °C, 72 h), five leaf bags being placed at each sampling station. The

bags were retrieved 30 days from the beginning of the experiment, placed in a plastic container separately, and rapidly brought to the laboratory. Here, leaves of each bag were gently washed with tap water to remove sediments and macroinvertebrate colonizers. All benthic macrofauna samples were managed following the same procedures described for benthic corer macrofauna.
Data on physiographic features of each ecosystem were provided by the partners involved in the project; chemical and physical water parameters (water salinity, dissolved oxygen and temperature) were monitored close to the bottom at each site during sampling, using a hand-held multi-probe meter (YSI 556).

3.3. *Data analysis*

Synthesis indices, namely the number of species/taxa, the Margalef richness index (d), the Shannon-Wiener diversity index (H', $\log_2$), were calculated per sample. The AMBI, M-AMBI, BITS and ISS indices were also calculated per individual sample. The AMBI index (Borja et al., 2000), is a frequent component of multimetric indices under scrutiny within the Ecological Quality Assessment of superficial waters in the European Union's Water Framework Directive (WFD). The multimetric M-AMBI index combines the species richness, the Shannon-Wiener diversity and the AMBI indices (Borja et al., 2003), using the software AMBI v4.1 (www.azti.es). The translation of the M-AMBI value into the Ecological Quality Statement (EQS) of the five WFD quality classes followed the boundaries mentioned in Muxika et al. (2007): 1.00 < High < 0.82 < Good < 0.62 < Moderate < 0.41 < Poor < 0.20 < Bad < 0. The index BITS (Benthic Index based on Taxonomic Sufficiency) has been developed specifically for coastal lagoons in the Mediterranean Sea (Mistri and Munari, 2008): 2.75 < High < 2.20 < Good < 1.65 < Moderate < 1.01 < Poor < 0.55 < Bad < 0 for sand; 2.30 < High < 1.84 < Good < 1.38 < Moderate < 0.92 < Poor < 0.46 < Bad < 0 for mud. It is written as $\log[(6fI + fII)/(fIII + 1) + 1] + \log[nI/(nII + 1) + nI/(nIII + 1) + 0.5nII/(nIII + 1) + 1]$ where fI is the sensitive families frequency, fII is the tolerant families frequency, and $fIII$ is the opportunistic families frequency. The ''+1" terms in the equation are needed in order to allow the division operation to be completed even when $fIII$ is null, and to prevent the eventuality of a log of zero if fI and fII are null. The translation of the

BITS value into the Ecological Quality Statement (EQS) of the five WFD quality classes followed the boundaries mentioned in Mistri and Munari (2008). Moreover, an Index of Size-spectra Sensitivity (ISS) (Basset et al., 2012) was calculated on the basis of the protocol of AMBI, applied to size spectra, using body size classes instead of species taxonomy and multiplying the relative proportion of each class for a sensitivity value assigned from a priori theoretically defined values of sensitivity $ISS = \Sigma p(CL_i)^* \omega_i$ where CL_i is the size class; ω_i is the sensitivity assigned value for the size class; and i is the size class descriptor, ranging from 1 to 6. The translation of the ISS value into the ecological quality statement (EQS) of the five WFD quality classes followed the boundaries mentioned in Barbone et al. (2012) 6.0 < High < 4.0 < Good < 2.8 < Moderate < 2.2 < Poor < 1.2 < Bad < 0. The benthic data obtained in the corer and the leaf-bag samples were compared in a model with sampling devices and salinity classes/habitat typologies as fixed and crossed factors, and with sampling sites, random and nested in lagoons, under the null hypothesis that no significant differences exist between the main factors and their interaction term. The salinity classes were set according to the classification defined in Barbone et al. 2012 for the same ecosystems; habitat typology were determined by the combination of the ecosystem bottom characteristics and the presence/absence of vegetation. Hypothesis testing was performed by Permutation Multivariate Analysis of Variance (PERMANOVA) (Anderson, 2001). This method allows partitioning the variability from a resemblance matrix and test individual terms, including interactions, using permutations (Anderson and ter Braak, 2003). The F-values in the main tests and the t-statistic in the pairwise comparisons were evaluated in terms of the significance among different groups, or levels, of the tested factor. Values of $P < 0.05$ reveal that the groups differ significantly. All data analysis used the software PRIMER v6 (Clarke and Gorley, 2006) with the add-on PERMANOVA+ (Anderson et al., 2008).

4. Results

Physiographic and physico-chemical features varied widely across the studied water bodies (Table 1). Surface area ranged from 0.3 km^2 in Grado fish farm (Itay) to 129.6 km^2 in Sinoe (Romania); depth ranged from 0.4 m in Margherita di Savoia to 1.1 m in Cesine and Alimini. Moreover, three ecosystems, Leahova, Sinoe and Cesine, were only temporarily connected to the sea; both outlet length and width were occasionally equal to zero, depending on freshwater pressures and wave action. The ecosystems were also differently characterised from submerged or emerged vegetation and from different types of bottom sediment. Physico-chemical parameters also varied considerably among ecosystems; the lowest water salinity (0.2%) was recorded in Cesine, Leahova and Sinoe, classified as oligohaline (salinity class=1), and the highest (64.8%) in Margherita di Savoia, classified as euhaline (salinity class=4). Two ecosystems, Grado fish farm and Narta were classified polyhaline-euhaline depending on the sampling sites.

Table 1. List of the studied transitional water ecosystems from North to South. Geographic position, main physiographic and chemical-physical characteristics are reported. Sediment: 1=mud; 2=sand. Salinity class: 1=oligohaline (<5 ‰); 2=mesohaline (6-18 ‰); 3=polyhaline (19-30‰); 4=euhaline (>30 ‰).Vegetation: 1=emerged vegetation; 2=absence of vegetation; 3=submerged vegetation. Typology: 1=sand without vegetation; 2=sand with submerged macrophytes; 3=sand with emerged macrophytes; 4= mud without vegetation; 5=mud with macroalgae; 6=mud with submerged macrophytes; 3=mud with emerged macrophytes.

Sites	Country	Coordinates		Surface	Depth	Vegetation	Sediment	Temperature	D. O.	Salinity class	Typology
		Latitude	Longitude	(km^2)	(m)			°C	(mg/l)	(‰)	
Grado fish farm	Italy	N 45.722°	E 13.362°	0.30	0.70	2; 3	1	23.2	6.8	3;4	4; 5
Grado Cavanata	Italy	N 45.712°	E 13.470°	2.08	1.00	1	1	21.7	9.7	3	7
Leahova	Romania	N 44.727°	E 29.028°	22.87	1.00	1; 2	1	18.5	7.8	1	4; 7
Sinoe	Romania	N 44.620°	E 28.888°	129.60	0.70	1; 2	1; 2	18.5	9.3	1	4; 7
Patok	Albania	N 41.635°	E 19.590°	7.09	0.60	2; 3	1	15.7	8.0	3	4; 6
Margherita di Savoia	Italy	N 41.429°	E 15.988°	11.98	0.40	2; 3	1	21.1	6.9	4	5; 6
Torre Guaceto	Italy	N 40.711°	E 17.795°	1.60	0.50	1; 3	1	19.8	3.3	2	6; 7
Narta	Albania	N 40.529°	E 18.426°	29.90	0.50	2	1	16.5	6.8	3;4	4
Cesine	Italy	N 40.358°	E 18.335°	0.93	1.10	1; 3	1; 2	19.9	8.3	1	6; 7
Alimini	Italy	N 40.202°	E 18.446°	1.38	1.10	1; 2	1; 2	17.9	7.1	3	1; 3; 4

Overall a total of 172 taxa were sampled in all ecosystems and using both samplers (Annex 1). Two taxa, *Corophium* sp. and *Gammarus insensibilis* (relative abundance 15.3% and 14.1%, respectively) constituted about 30% of abundance of the benthic macrofauna, 14 taxa showed a relative abundance ranging between 10% and 1% and 38 taxa showed a relative abundance more than 0.1% while the other taxa were found with very few individuals. Fourty nine taxa were found only in sediment samples and fifty two only in leaf bags; 71 were common to both samplers. This indicates similar overall taxa richness both in sediment and leaf-bag samples.

Accounting for corer and leaf-bag samples separately, *Corophium* sp., *Dreissena* sp. and *Ventrosia ventrosa* showed a mean abundance per site higher than 100 individuals, in Alimini, Leahova and Torre Guaceto, in the corer samples; three taxa (*Corophium plumosus*, Oligochaeta and *Ventrosia ventrosa*) showed a mean abundance higher than 50 individuals per site in Cesine, Sinoe and Margherita di Savoia; while most of taxa were rare with a mean abundance equal to or slightly higher than 1 individual per sampling site (Table 2). In leaf bag samples, the most abundant taxa (*Lekanesphaera monodi*, Gammaridae spp., *Corophium* sp., *Corophium salinarius*) were registered for Margherita di Savoia with a mean abundance more than 50 individuals per site; *L. monodi* showed a high abundance also in Alimini, while few individuals on average were sampled for the other taxa in all ecosystems (Table 3).

The relative distribution of specimens per major taxonomic group, per sampler in all studied ecosystems, is shown in Figure 2. Arthropods were a dominant component of benthic communities in both box corer and leaf bags, molluscs were similarly represented in both samplers, while annelids characterized mainly the corers. Similarly, the distinction between the two sampling devices is evident when the functional feeding groups are considered. In the sediment samples, most invertebrates were detritivorous-collectors, followed by the scrapers and shredders; on the other hand, in the leaf bags about 50% of the benthic macroinvertebrates were detritivorous-shredders and the other 50% was equally distributed between detritivorous-scrapers and collectors (Figure 3).

Table 2. Mean abundance per site of each taxon sampled in all studied ecosystem, in the box-corer samples, ranked from highest to lowest.

G. fish farm		G. Cavanata		Leahova		Sinoe		Patok	
Perioculodes aequimanus	6.2	*Corophium* sp.	24.7	*Dreissena* sp.	118.5	Oligochaeta [n.d.]	55.0	*Gammarus* sp.	15.3
Corophium sp.	6.0	Chironomidae spp.	13.3	Oligochaeta [n.d.]	28.9	Cumacea [n.d.]	14.4	Tubificidae spp.	9.0
Abra segmentum	4.3	*Gammarus aequicauda*	3.8	*Chironomus* sp.	13.8	*Corophium* sp.	11.7	*Scrobicularia plana*	8.7
Acanthocardia echinata	2.9	*Acanthocardia echinata*	1.0	*Corophium* sp.	7.4	*Chironomus* sp.	5.1	Nephtyidae spp.	5.5
Haminoea navicula	2.4	*Abra segmentum*	1.0	*Batracobdella* sp.	6.0	Coleoptera [n.d.]	5.0	Isopoda [n.d.]	4.9
Elasmopus sp.	1.7	*Capitella capitata*	1.0	Naticidae spp.	6.0	Diamesinae spp.	3.2	Polychaeta [n.d.]	4.5
Ampelisca diadema	1.5	*Malacoceros fuliginosus*	1.0	*Caenis* sp.	5.0	Tanypodinae	2.8	Haplotaxidae spp.	4.3
Loripes lacteus	1.5	*Nereis* sp.	1.0	Valvatidae spp.	5.0	*Dreissena* sp.	1.8	*Chironomus* sp.	3.0
Abra prismatica	1.3			*Erpobdella* sp.	4.5	Ecnomidae spp.	1.7	Lumbricidae spp.	3.0
Cirratulus sp.	1.1			*Planorbarius* sp.	4.0	Ceratopogonidae spp.	1.3	Nereididae spp.	2.8
Caprella acanthifera	1.0			Tanypodinae spp.	3.9	*Gammarus* sp.	1.3	Syllidae spp.	2.0
Capitella capitata	1.0			*Asellus* sp.	3.0	*Acentria* sp.	1.0	*Cerastoderma glaucum*	1.3
Cyclope neritea	1.0			*Ventrosia ventrosa*	3.0	*Caenis* sp.	1.0	*Crangon crangon*	1.0
Dexamine spinosa	1.0			Ecnomidae spp.	2.0	Chironomidae spp.	1.0	Enoplida [n.d.]	1.0
Gammaridae spp.	1.0			*Ephemerella* sp.	2.0	Diptera [n.d.]	1.0	Nematoda [n.d.]	1.0
Harpinia crenulata	1.0			Lepidoptera [n.d.]	2.0	Nematomorpha	1.0	Oligochaeta [n.d.]	1.0
Hediste diversicolor	1.0			*Lestes* sp.	2.0	Sabellidae spp.	1.0		
Heteromastus filiformis	1.0			Diamesinae spp.	1.8				
Hemiptera [n.d.]	1.0			Nematomorpha [n.d.]	1.6				
Iphinoe serrata	1.0			*Asellus aquaticus*	1.5				
L. arcuatus	1.0			Chironomidae spp.	1.5				
Malacoceros fuliginosus	1.0			Cumacea [n.d.]	1.3				
Nephtys hombergii	1.0			Coleoptera [n.d.]	1.3				
Nassarius reticulatus	1.0			*Acentria* sp.	1.0				
Nereis sp.	1.0			Ceratopogonidae spp.	1.0				
Notomastus sp.	1.0			Criodrilidae spp.	1.0				
Oligochaeta [n.d.]	1.0			Diptera [n.d.]	1.0				
Opheliidae spp.	1.0			*Gammarus insensibilis*	1.0				
Polydora ciliata	1.0			*Gammarus* sp.	1.0				
Prionospio cirrifera	1.0			Leptoceridae spp.	1.0				
Paraonidae spp.	1.0			*Piscicola* sp.	1.0				
Sternaspis scutata	1.0			*Planorbis* sp.	1.0				
Streblospio shrubsolii	1.0			Trichoptera [n.d.]	1.0				
Trachythyone elongata	1.0								
Tanaidacea [n.d.]	1.0								

Table 2. Continued.

M. di Savoia		T. Guaceto		Narta		Cesine		Alimini	
Ventrosia ventrosa	76.1	*Ventrosia ventrosa*	167.6	*Chironomus* sp.	10.3	*Chironomus plumosus*	52.5	*Corophium* sp.	210.5
Corophium sp.	49.4	*Bithynia tentaculata*	20.8	*Gammarus* sp.	5.4	*Ficopomatus enigmaticus*	17.0	Oligochaeta [n.d.]	29.2
Chironomus salinarius	24.4	*Chironomus plumosus*	12.9	*Cerastoderma glaucum*	3.2	*Gammarus insensibilis*	14.9	*Loripes lacteus*	14.7
Microdeutopus gryllotalpa	23.3	*Gammarus insensibilis*	8.1	Lumbricidae spp.	3.0	*Ventrosia ventrosa*	6.8	*Microdeutopus gryllotalpa*	12.3
Gammarus insensibilis	23.1	*Lekanesphaera hookeri*	2.0	Naidinae spp.	2.0	Psychomyiidae spp.	6.0	*Chironomus plumosus*	9.3
Loripes lacteus	18.9	Tabanidae spp.	1.7	Phyllodocidae spp.	2.0	*Lekanesphaera hookeri*	3.2	Enchytraeidae spp.	7.0
Microdeutopus sp.	15.0	*Theodoxus fluviatilis*	1.6	Tubificidae spp.	1.5	*Hediste diversicolor*	3.1	*Limnoria lignorum*	7.0
Oligochaeta [n.d.]	13.5	Diamesinae spp.	1.5	Haplotaxidae spp.	1.0	Chironomidae spp.	2.6	*Tanais dulongii*	6.8
Polychaeta [n.d.]	7.8	Chironomidae spp.	1.3			*Cerastoderma glaucum*	2.3	*Bittium reticulatum*	4.2
Lumbrineridae spp.	6.7	*Aeshna mixta*	1.0			Diamesinae spp.	1.0	Chironomidae spp.	4.0
Cerastoderma glaucum	5.2	Asellidae spp.	1.0			*Nymphula nymphaeata*	1.0	*Ventrosia ventrosa*	4.0
Naineris laevigata	4.5	Ceratopogonidae spp.	1.0			Tanypodinae spp.	1.0	*Gammarus insensibilis*	3.0
Tellina sp.	4.3	*Hediste diversicolor*	1.0					*Nassarius* sp.	2.5
Abra segmentum	3.5	*Planorbarius corneus*	1.0					*Abra segmentum*	2.1
Spionidae spp.	3.1	Stratiomyidae spp.	1.0					Diamesinae spp.	2.0
Chironomidae spp.	3.1	Tanypodinae spp.	1.0					*Hediste diversicolor*	1.8
Actiniaria [n.d.]	3.1	Tipulidae spp.	1.0					*Mytilaster* sp.	1.7
Cerithium sp.	3.0	Trichoptera [n.d.]	1.0					*Cerastoderma glaucum*	1.4
Diamesinae spp.	2.8							*Glycera* sp.	1.1
Nereididae spp.	2.3							*Cyclope neritea*	1.0
Dexamine sp.	2.0							*Dosinia lupinus*	1.0
Nematoda [n.d.]	2.0							*Ficopomatus enigmaticus*	1.0
Haminoea sp.	1.5							*Gastrana fragilis*	1.0
Mysidae	1.3							Tanypodinae spp.	1.0
Ophiuridae	1.3								
Artemia salina	1.0								
Ceratopogonidae	1.0								
Diptera [n.d.]	1.0								
Gammaridae	1.0								
Hediste diversicolor	1.0								
Idotea balthica	1.0								
Lekanesphaera monodi	1.0								
Lepidoptera [n.d.]	1.0								
Nassarius sp.	1.0								
Planariidae	1.0								
Stratiomyidae	1.0								
Tapes sp.	1.0								

Table 3. Mean abundance per site of each taxon sampled in all studied ecosystem, in the leaf-bag samples, ranked from highest to lowest.

G. fish farm		G. Cavanata		Leahova		Sinoe		Patok	
Melita palmata	12.4	*Ventrosia ventrosa*	9.6	*Echinogammarus* sp.	42.5	*Chironomus* sp.	12.8	*Gammarus* sp.	13.1
Paludinella littorina	10.3	*Corophium* sp.	5.8	*Dreissena* sp.	19.2	Oligochaeta [n.d.]	11.7	Isopoda [n.d.]	3.1
Cumacea [n.d.]	6.8	*Gammarus insensibilis*	5.6	*Asellus* sp.	13.0	*Corophium* sp.	5.6	*Ventrosia ventrosa*	2.2
Perinereis cultrifera	3.9	*Chironomus salinarius*	2.3	Amphipoda [n.d.]	7.3	*Echinogammarus* sp.	4.8	Tubificidae spp.	2.2
Ventrosia ventrosa	3.5	*Haminoea* sp.	2.1	*Corophium* sp.	7.0	Gammaridae spp.	3.0	Nereididae spp.	2.1
Tubificidae spp.	3.0	Actiniaria [n.d.]	1.0	Gammaridae spp.	7.0	Gastropoda [n.d.]	3.0	*Hydrobia acuta*	2.0
Haminoea sp.	2.8	*Cerastoderma glaucum*	1.0	*Caenis* sp.	5.7	*Caenis* sp.	2.8	Haplotaxidae spp.	1.5
Portunidae spp.	2.1	Cumacea [n.d.]	1.0	*Bithynia* sp.	4.0	*Dreissena* sp.	1.3	*Cerastoderma glaucum*	1.3
Amphipoda [n.d.]	2.0	*Idotea balthica*	1.0	*Gammarus* sp.	4.0	Ecnomidae spp.	1.3	*Cyclope neritea*	1.3
Eulimella ventricosa	2.0	*Idotea* sp.	1.0	*Chironomus* sp.	3.4	*Asellus* sp.	1.0	*Scrobicularia plana*	1.0
Pirenella sp.	2.0	Oligochaeta [n.d.]	1.0	Valvatidae spp.	3.3	Ceratopogonidae spp.	1.0	Syllidae sp.	1.0
Chironomus salinarius	1.6			*Gammarus insensibilis*	3.0	Chironomidae spp.	1.0		
Tapes sp.	1.3			Oligochaeta [n.d.]	2.6	Cumacea [n.d.]	1.0		
Cerastoderma glaucum	1.3			Hydrobiidae spp.	2.0	Diptera [n.d.]	1.0		
Abra segmentum	1.0			*Planorbarius* sp.	2.0	*Gammarus* sp.	1.0		
Bittium reticulatum	1.0			Ecnomidae spp.	1.5	*Piscicola* sp.	1.0		
Cyathura carinata	1.0			*Erpobdella* sp.	1.4	Tanypodinae spp.	1.0		
Corophium sp.	1.0			*Helobdella* sp.	1.3				
Diamesinae spp.	1.0			Lepetidae spp.	1.3				
Gammarus insensibilis	1.0			Ancylidae spp.	1.0				
Gastropoda [n.d.]	1.0			*Batracobdella* sp.	1.0				
Loripes lacteus	1.0			Diamesinae spp.	1.0				
Littorina neritoides	1.0			Gastropoda [n.d.]	1.0				
Oligochaeta [n.d.]	1.0			*Glossiphonia* sp.	1.0				
				Nematomorpha [n.d.]	1.0				
				Physidae spp.	1.0				
				Piscicola sp.	1.0				
				Planorbis sp.	1.0				
				Tanypodinae spp.	1.0				

Table 3. Continued.

M. di Savoia		T. Guaceto		Narta		Cesine		Alimini	
Lekanesphaera monodi	92.3	*Chironomus plumosus*	28.5	*Gammarus* sp.	6.7	*Gammarus insensibilis*	38.4	*Lekanesphaera monodi*	81.7
Gammaridae spp.	59.4	*Ventrosia ventrosa*	24.7	*Chironomus* sp.	3.6	*Ventrosia ventrosa*	35.3	*Corophium* sp.	45.9
Corophium sp.	58.6	*Gammarus insensibilis*	17.3	Lumbricidae spp.	2.0	*Lekanesphaera hookeri*	35.2	*Pirenella* sp.	42.9
Chironomus salinarius	54.7	*Lekanesphaera hookeri*	7.0	Tubificidae spp.	1.9	*Chironomus plumosus*	24.1	*Tanais dulongii*	19.0
Ventrosia ventrosa	31.4	*Aeshna mixta*	1.0	*Ventrosia ventrosa*	1.8	*Lekanesphaera monodi*	9.0	*Microdeutopus gryllotalpa*	13.1
Cerastoderma glaucum	31.4	*Bithynia tentaculata*	1.0	*Hydrobia acuta*	1.8	*Ficopomatus enigmaticus*	2.1	*Melita palmata*	8.0
Microdeutopus gryllotalpa	15.5	Ceratopogonidae spp.	1.0	Naididae spp.	1.4	Oligochaeta [n.d.]	2.0	*Microdeutopus* sp.	6.8
Gammarus insensibilis	15.3	Chironomidae spp.	1.0	Phyllodocidae spp.	1.3	Chironomidae sp.	1.2	*Pusillina* sp.	6.8
Dexamine sp.	14.0	*Lestes* sp.	1.0	Haplotaxidae spp.	1.3	*Bittium reticulatum*	1.0	Oligochaeta [n.d.]	6.3
Artemia salina	10.5	*Nereis diversicolor*	1.0	Nereididae spp.	1.2	*Bithynia tentaculata*	1.0	Ophiuridae spp.	5.6
Microdeutopus sp.	10.4	*Planorbarius corneus*	1.0	*Cerastoderma glaucum*	1.0	*Mytilaster* sp.	1.0	Porcellanidae spp.	4.8
Gammarella fucicola	9.0	*Theodoxus fluviatilis*	1.0	*Cyclope neritea*	1.0	*Nereis diversicolor*	1.0	*Mytilaster* sp.	4.7
Jaera hopeana	7.5	Tabanidae spp.	1.0	Nephtyidae spp.	1.0	Odonata [n.d.]	1.0	*Chironomus plumosus*	4.0
Microdeutopus anomalus	7.4	Tanypodinae spp.	1.0	*Scrobicularia cottardi*	1.0	Stratiomyidae spp.	1.0	*Venerupis* sp.	4.0
Mysidae spp.	7.0							*Ventrosia ventrosa*	3.9
Ophiuridae spp.	5.5							*Bittium reticulatum*	3.9
Diamesinae spp.	5.0							*Idotea balthica*	3.8
Anisus sp.	4.0							Nereididae spp.	3.8
Loripes lacteus	4.0							*Gammarus insensibilis*	3.5
Oligochaeta [n.d.]	3.7							*Cerastoderma glaucum*	3.2
Lumbrineridae spp.	3.5							Nematoda [n.d.]	3.1
Spionidae spp.	3.5							*Lekanesphaera hookeri*	3.0
Bithynia tentaculata	3.3							Mysidae spp.	2.8
Pusillina sp.	3.0							Cardiidae spp.	2.5
Nereididae spp.	2.6							*Dosinia lupinus*	2.0
Gibbula adansonii	2.3							*Mytilus galloprovincialis*	2.0
Actiniaria [n.d.]	2.3							*Nereis diversicolor*	2.0
Haminoea sp.	2.0							Turbellaria [n.d.]	2.0
Leucothoe sp.	2.0							Polychaeta [n.d.]	1.7
Nereis diversicolor	2.0							Rissoidae spp.	1.5
Tricladida [n.d.]	2.0							Sabellidae spp.	1.5
Chironomidae spp.	1.5							Gammaridae spp.	1.3
Diptera [n.d.]	1.5							Platyhelmintes [n.d.]	1.3
Nassarius sp.	1.5							*Abra segmentum*	1.0
Polychaeta [n.d.]	1.5							*Balanus improvisus*	1.0
Sphaeromatidae spp.	1.4							Brachyura [n.d.]	1.0
Idotea balthica	1.3							*Carcinus maenas*	1.0
Naineris laevigata	1.3							*Cyclope neritea*	1.0
Abra segmentum	1.0							Cirratulidae spp.	1.0
Amphipoda [n.d.]	1.0							Gastropoda [n.d.]	1.0
Asellidae spp.	1.0							*Loripes lacteus*	1.0
Bittium reticulatum	1.0							*Littorina neritoides*	1.0
Bivalvia [n.d.]	1.0							*Nassarius* sp.	1.0
Ephydridae spp.	1.0							Nemertina	1.0
Flabellina sp.	1.0							Planariidae spp.	1.0
Melita palmata	1.0							*Streptosyllis* sp.	1.0
Perinereis cultrifera	1.0							*Tapes* sp.	1.0

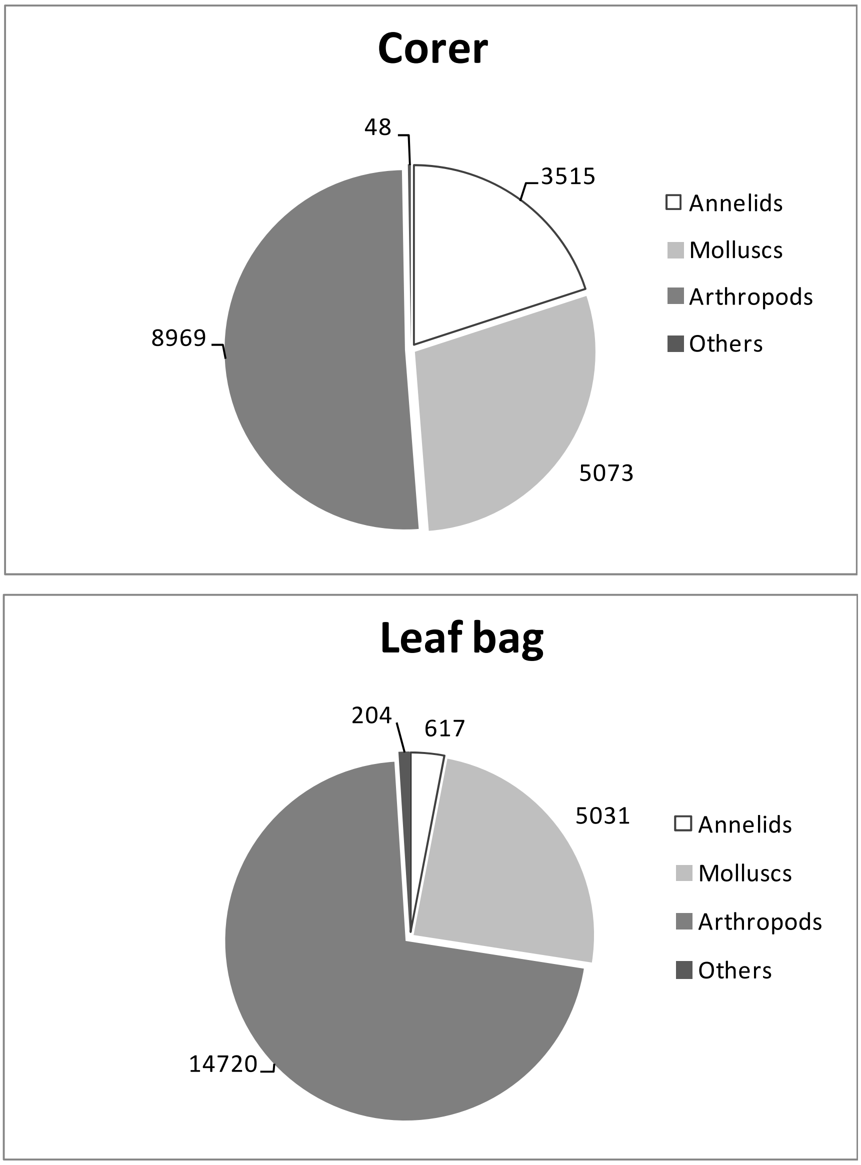
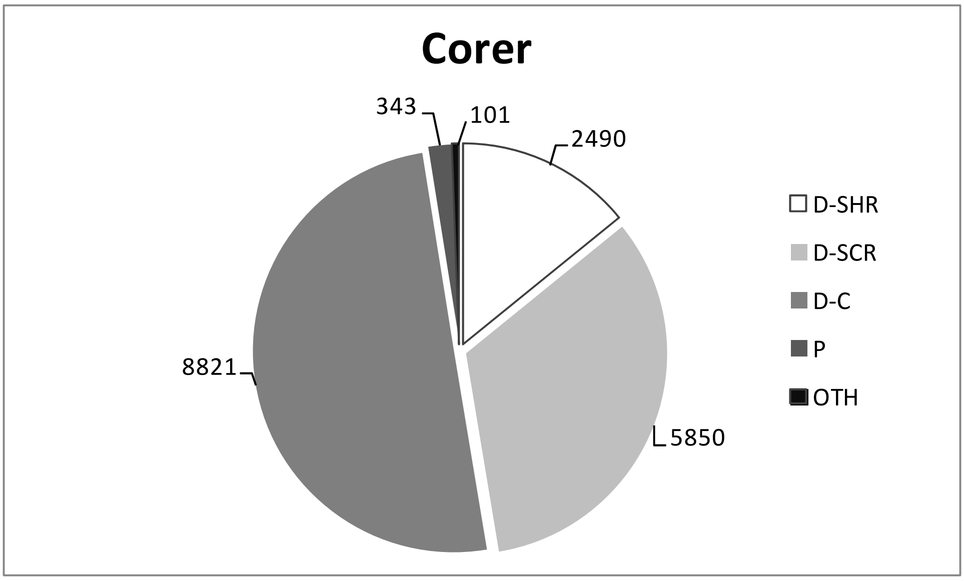


Figure 2. Pie charts representing the proportion of Anellids, Molluscs, Arthropods and Other groups in leaf-bag samples and corer sediment samples. The values close to each pie chart indicate total abundance.



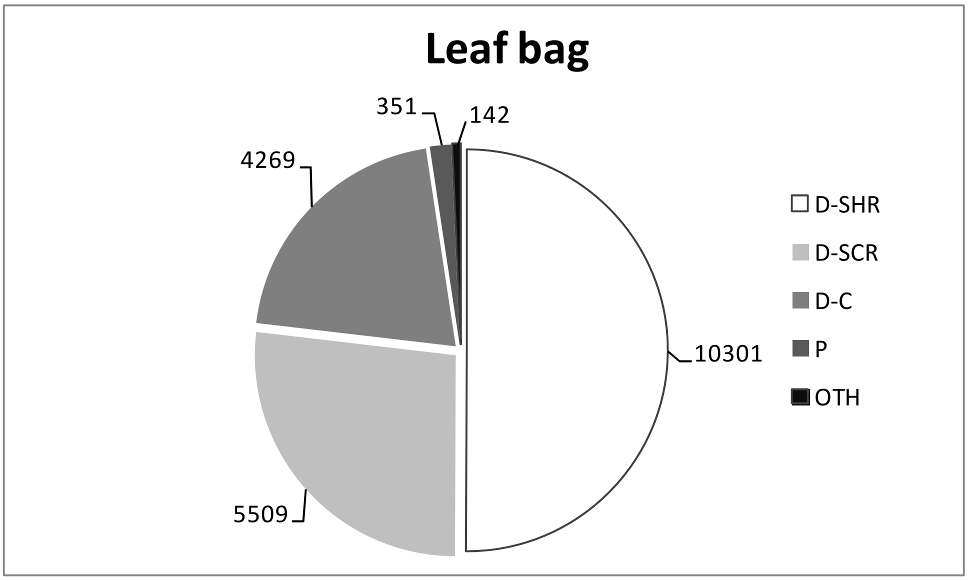


Figure 3. Pie charts representing the proportion of Functional Feeding Groups in leaf-bag samples and corer sediment samples. The values close to each pie chart indicate total abundance. (D-SH=detritivorous shredders, D-SC=detritivorous scrapers, D-C=detritivorous filtering or gathering collectors, P=predators, OTH=others).

These differences in terms of dominant phylum, corer and leaf-bag samples did not show significant correlation for the main biotic indices even if were similar for the number of taxa and individuals (S: $r = 0.671$, d.f. = 8, $P < 0.05$; N: $r = 0.754$, d.f. = 8, $P < 0.05$; d: $r = 0.562$, d.f. = 8, n.s.; H': $r = 0.594$, d.f. = 8, n.s.) (Figure 4).

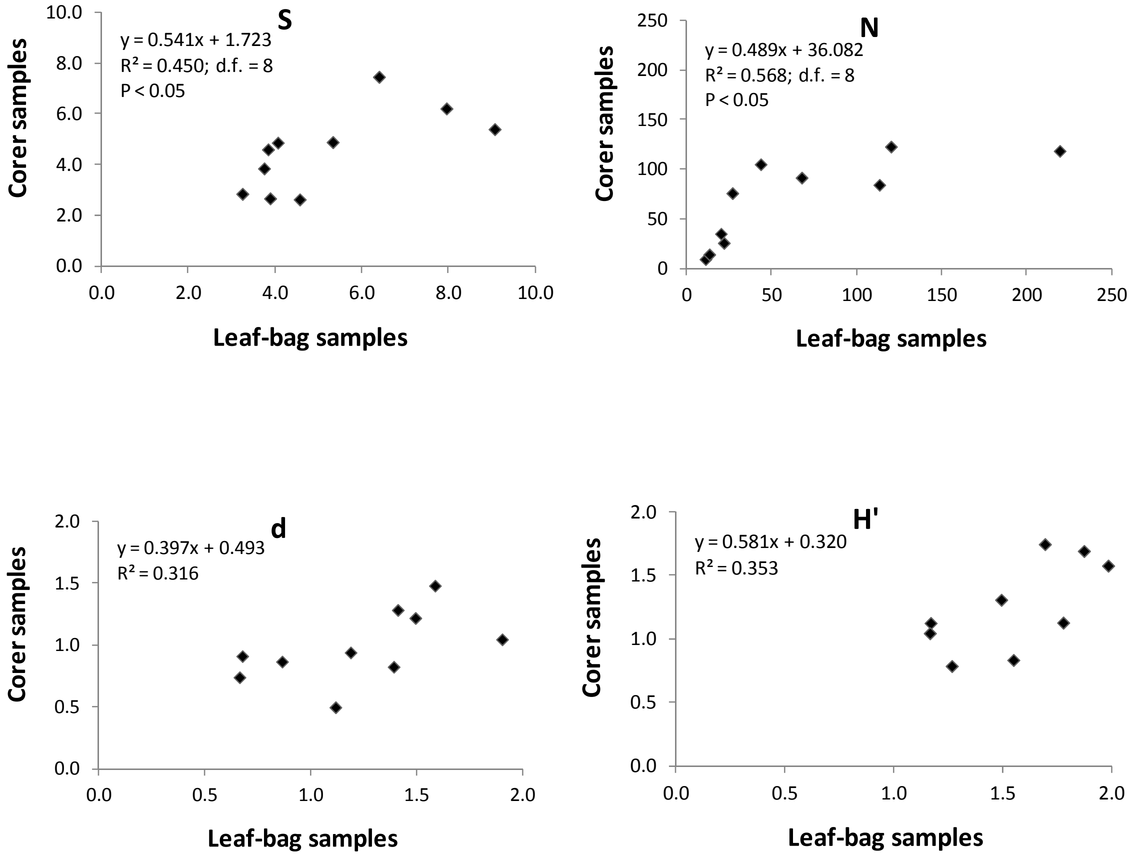


Figure 4. Analysis of correlation between corer samples and leaf-bag samples of main biotic indices (S = number of taxa; N = number of individuals; d = taxa richness; H' = diversity) calculated per individual sample.

The set of graphs in Figure 5 presents the trend of the number of taxa (S) and diversity related indices (d and H') among the salinity classes both for corer and leaf-bag samples. From oligohaline to euhaline classes, the number of taxa, Margalef index and Shannon index (H') showed a regular trend, increasing from oligohaline to euhaline ecosystems, with the exception of mesohaline class where the lowest values was observed; moreover, Shannon index showed higher values for leaf-bag with respect to corer samples through all salinity classes. A comparable result was evident with the mean Bray-Curtis similarity per salinity class and sampling device, following a range of data transformation (Table 4). Both with the corer and leaf-bag samples, the mean Bray-Cutis similarity increased with diminishing water salinity (with diminishing taxa richness and diversity), with the exception of mesohaline area where the similarity was the highest, and this was particularly evident for leaf bags.

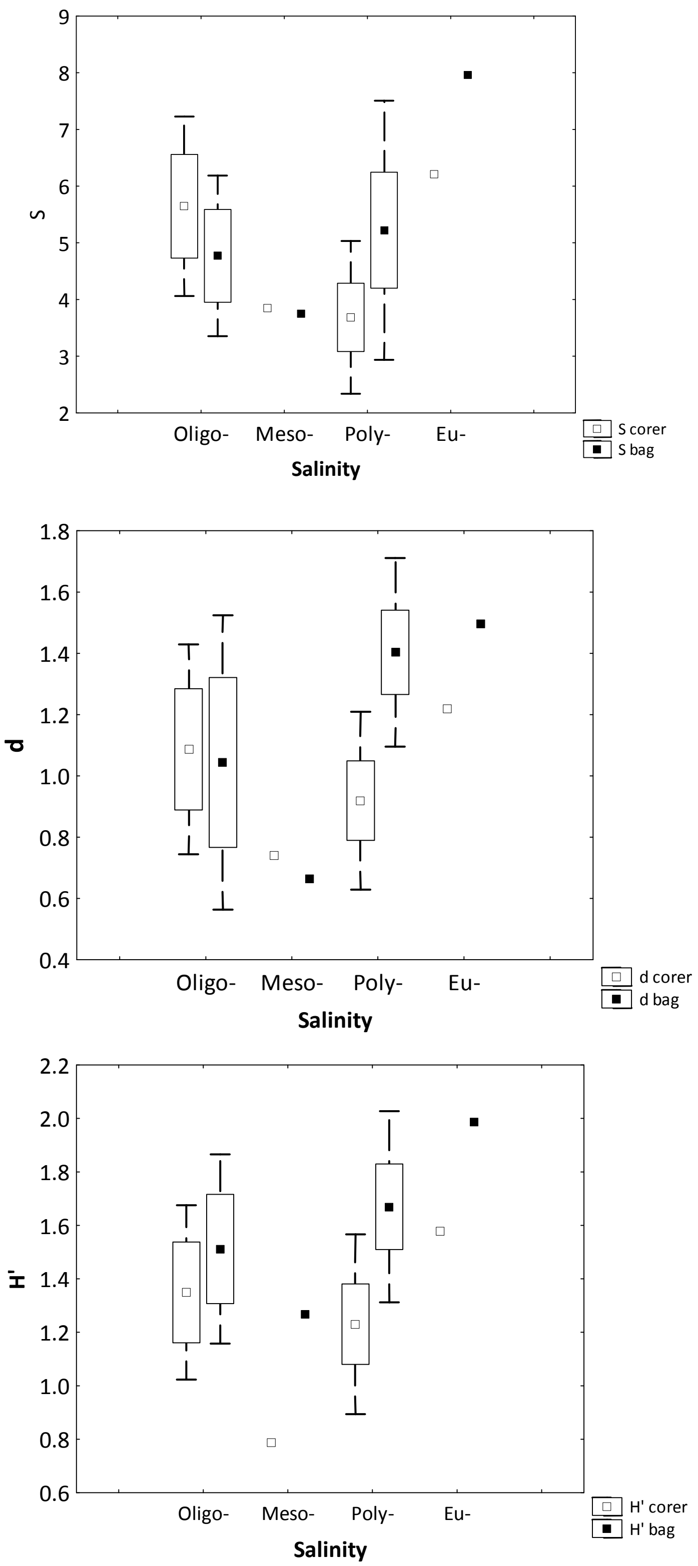


Figure 5. Box Plot (mean; box: mean + s.e.; whisker: mean + s.d.) of number of taxa (S) and related biotic indices (Margalef [d] and Shannon-Wiever [H']) grouped by salinity class and calculated on the corer and leaf-bag data.

Table 4. Mean Bray-Curtis percent similarity within salinity class, in the corer and leaf-bag samples, following data transformation (square root).

	oligohaline	mesohaline	polyhaline	euhaline
box corer	59.10	44.79	45.16	29.06
leaf bag	49.15	64.57	50.54	31.09

The inter-site Bray-Curtis similarity matrix was also submitted to hypothesis testing under the null hypothesis that no difference exists between sampling devices and salinity classes. A four factor nested model was used, with sampling devices and salinity classes as fixed and crossed factors, lagoons nested in salinity class and sampling sites nested in lagoons. The null hypothesis was rejected for the sampling devices (pseudo-F = 1.991, P = 0.049), while it was accepted for the salinity (pseudo-F = 1.119, P = 0.304) (Table 5A). On the other hand, salinity resulted a significant factor when considered at ecosystem level, among lagoons nested in salinity (pseudo-F = 8.052, P < 0.0001), and highlighted also a significant interaction term: sampling device x lagoon nested in salinity (pseudo-F = 3.605, P < 0.0001) (Table 5A). Pairwise comparisons between samplers within each salinity class showed consistent results: the benthic communities were not different between the two sampling devices within salinity class (t = 1.19, P = 0.26 for oligohaline; t = 1.52, P = 0.12 for mesohaline; t = 1.07, P = 0.35 for polyhaline; t = 1.28, P = 0.09 for euhaline), whereas benthic macrofauna communities resulted different between the two sampling devices within lagoon nested in salinity in all cases with exception of Torre Guaceto (P = 0.12) and Margherita di Savoia (P = 0.08).

The same model was used to test the null hypothesis that no difference exists between sampling devices and habitat typologies. The null hypothesis was rejected for the sampling devices (pseudo-F = 4.257, P = 0.0009), whereas it was accepted for the habitat typology (pseudo-F = 1.222, P = 0.176) (Table 5B). Typology resulted significant when considered at ecosystem level, among lagoons nested in typology (pseudo-F = 5.495, P = 0.0001) and in the interaction term [sampling device x lagoon nested in habitat typology] (pseudo-F = 2.684, P = 0.0001) (Table 5B). Pairwise comparisons between samplers within each habitat typology showed different results depending on the considered typology: the benthic communities were different between the two samplers in the case of typology 'mud without vegetation' (t = 1.43, P = 0.03) and for 'sand with submerged macrophytes' (t = 5.55 P = 0.01); whereas benthic macrofauna was different between the two sampling devices within lagoon nested in typology only in any lagoons but without a clear pattern.

Table 5. Results of PERMANOVA 4-ways design **A:** (sampler[sam], salinity[sal], lagoon[la], sampling site[si]) and **B:** (sampler[sam], typology [Ty], lagoon[la], sampling site[si])performed on Bray-Curtis similarity matrix of corer and leaf-bag macrofauna data.

A Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Sam	1	30082	30082	1.991	0.0492	9926	0.0281
Sal	3	1.65E+09	55162	1.119	0.3047	9900	0.3132
La(Sal)	8	4.26E+09	53295	8.052	0.0001	9832	0.0001
SamxSal	3	31694	10565	0.717	0.8891	9878	0.8983
Si(La(Sal))	35	2.49E+09	7113	8.148	0.0001	9575	0.0001
SamxLa(Sal)	8	1.25E+09	15662	3.605	0.0001	9822	0.0001
SamxSi(La(Sal))	35	1.63E+09	4643	5.319	0.0001	9582	0.0001
Res	369	3.22E+09	873				
Total	462	1.62E+10					

B Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Sam	1	42598	42598	4.257	0.0009	9926	0.0002
Ty	6	2.18E+09	36328	1.222	0.1760	9883	0.1254
La(Ty)	14	4.82E+08	34393	5.495	0.0001	9813	0.0001
SamxTy	6	63911	10652	1.077	0.3378	9868	0.3139
Si(La(Ty))	26	1.64E+09	6309,7	7.228	0.0001	9640	0.0001
SamxLa(Ty)	14	1.54E+09	10990	2.684	0.0001	9798	0.0001
SamxSi(La(Ty))	26	1.07E+09	4125,7	4.726	0.0001	9652	0.0001
Res	369	3.22E+09	873,01				
Total	462	1.62E+10					

The benthic data obtained with both sampling devices were also used for the calculation of ecological quality assessment indices AMBI, M-AMBI, BITS and ISS, the mean values of which are shown in Table 6, per ecosystem and sampling device.

The data of biotic indices was submitted to hypothesis testing, under the null hypothesis that no significance differences exist in the indices between sampling devices and among salinity classes/habitat typology. A four factor model was used, with samplers and salinity classes/habitat typology as fixed factors, lagoons nested in salinity/habitat typology and sampling sites nested in lagoons. The null hypothesis was rejected for samplers (pseudo-F = 4.614, p = 0.020) and accepted for salinity (pseudo-F = 0.657, p = 0.719); whereas, the interaction term [lagoon x salinity classes] was significant (pseudo-F = 6.765, p = 0.0001). The pairwise comparisons between the two samplers was made for each salinity class and was found not significantly different for all classes (t = 0.97, p = 0.46 for oligohaline; t = 1.09, p = 0.39 for mesohaline; t = 1.70, p = 0.07 for polyhaline; t = 0.99, p = 0.40 for euhaline); in the same way, consistent results was observed when the comparisons were repeated for each lagoon nested in salinity. On the other hand, the null hypothesis was accepted both for samplers (pseudo-F = 1.316, p = 0.255) and habitat typology (pseudo-F = 0.687, p = 0.753).

When translation the biotic index values into quality statements, the leaf-bag samples were classified one class above with respect to sediment samples in the most of cases both for M-AMBI and BITS, whereas for the ISS index the results were similar both for sediment and leaf-bag samples (cf. Table 6).

Table 6. Mean values for the AMBI, M-AMBI, BITS and ISS indices per sampling device calculated for each studied ecosystem. Ecosystems are grouped and listed following the salinity classes from oligohaline to euhaline. EQS: Ecological Quality Statement.

	AMBI		M-AMBI	EQS	M-AMBI	EQS	BITS	EQS	BITS	EQS	ISS	EQS	ISS	EQS
	box corer	leaf bag	box corer		leaf bag		box corer		leaf bag		box corer		leaf bag	
Cesine	2.57	2.22	0.58	Moderate	0.61	Moderate	0.85	Poor	2.33	High	2.39	Moderate	1.72	Poor
Leahova	4.71	3.18	0.66	Good	0.82	Good	0.18	Bad	1.17	Moderate	2.30	Moderate	2.53	Moderate
Sinoe	5.47	4.00	0.31	Poor	0.49	Moderate	0.58	Poor	0.79	Poor	1.80	Poor	1.61	Poor
T. Guaceto	2.74	2.44	0.50	Moderate	0.58	Moderate	0.74	Poor	0.98	Moderate	1.58	Poor	1.49	Poor
Alimini	3.50	2.65	0.55	Moderate	0.91	High	2.31	High	2.76	High	2.57	Moderate	2.70	Moderate
G. Cavanata	2.68	2.28	0.53	Moderate	0.66	Good	0.75	Poor	2.41	High	1.14	Bad	1.58	Poor
Patok	2.10	0.99	0.69	Good	0.76	Good	2.21	Good	2.21	High	3.16	Good	1.72	Poor
Narta	3.11	1.72	0.50	Moderate	0.73	Good	0.93	Moderate	1.84	Good	1.74	Poor	2.03	Poor
G. fish farm	2.41	2.93	0.92	High	0.64	Good	1.64	Good	1.78	Good	1.42	Poor	1.20	Bad
M. di Savoia	2.85	2.27	0.67	Good	0.83	High	2.00	Good	2.52	High	2.67	Moderate	2.77	Moderate

5. Discussions

The selection of the sampling methodology is crucial in every monitoring program, but we know little about the performance of different sampling techniques applied in aquatic ecosystems among which transitional aquatic ecosystems. The goal for comparing communities sampled by different strategies is to have sampling techniques that best capture samples, representative of the community. All methods have biases and problems associated with them and a number of factors such as substrate type, presence of emergent vegetation and depth can influence the usefulness of different techniques. The effect of sampling technique on the characterization of the macroinvertebrate community has been observed in several works carried out in aquatic ecosystems (Somerfield and Clarke, 1997).

In the present study, the benthic macroinvertebrate communities obtained with two different sampling techniques are described and compared. The comparison is made between sediment and trap technique, more exactly hand-held corer and leaf bags, focusing on the composition and patterns of macroinvertebrate fauna among any given ecosystems distributed on a large spatial scale.

The quantitative and semiquantitative (leaf-bag) sampling methods provided different results about the species composition and structural characteristics of benthic communities even if the observed patterns were similar. The statistical multivariate analysis performed on all data set evidenced significant differences between the two devices about the sampled communities.

As regards the first main result, some information on this subject and, more exactly about the comparison of sampling techniques of benthic macrofauna, has been provided for lotic systems (Stein et al., 2008; Alonso and Camargo, 2010), lakes (Tolonen and Hamalainen, 2010) and ponds (Garcìa-Criado and Trigal, 2005), wetlands (Cheal et al., 1993); on the other hand, only one work considering and comparing different techniques to sample benthic macrofauna is available for transitional aquatic ecosystems (Quintino et al., 2011).

Some of those studies highlighted several biases and methodological problems associated with sampling devices and procedures. Since it is known that benthic macroinvertebrate community composition and distribution in aquatic environments is closely linked to habitat patchiness and species requirements (Tolonen et al., 2001), the different sampling techniques used in this study sampled different benthic macrofauna assemblages both in terms of species composition and functional groups. Obtained results highlighted differences in terms of relative distribution of specimens per major taxonomic group and of relative distribution per functional feeding group. Benthic communities obtained through the hand-held corer were characterised by annelids almost absent in leaf-bag samples, highlighting a selectivity for infauna; on the other hand, macroinvertebrate assemblages colonising leaf bags were constituted in high percentage by arthropods highlighting the selectivity of this technique for epifauna. This evidence was in agreement with the results obtained for Ria de Aveiro in Portugal where significant differences were observed in terms of diversity and functional groups of benthic macrofauna colonising leaf bags and macrofauna present in the sediment samples (Quintino et al., 2011). Similarly, benthic macrofauna sampled through the corer was constituted mainly by scrapers and collectors, whereas the macrofauna obtained through leaf bags were characterised mainly by shredders. As suggested in other works, these results could be explained in terms of attractiveness of leaf bags which constitute in the ecosystem an additional supply of food mainly for shredder guilds (Cortes et al., 1997; Sangiorgio et al., 2010).

On the eco-regional scale of the studied ecosystems, the salinity groups and habitat typology did not result the forcing factors determining the differences of benthic assemblages sampled through the two sampling devices. This observation contrasts the results obtained in Ria de Aveiro where the strong salinity gradient characterising the studied areas resulted the most important factor influencing benthic macroinvertebrate communities and this effect was also related to the different sampling techniques. Probably, in our study the heterogeneity among lagoons was more important than the considered ecotopes (e.g., salinity groups), and this could be mean that the expression, for example, of the salinity gradient depends on the ecosystem. This observation is confirmed by the significant differences observed when the analysis was performed among the ecosystems nested in the salinity groups. In the same way, the habitat type and substrate that in other works resulted ecosystem characteristics influencing both the species and the density of macroinvertebrates occupying a site (Muzzafar and Colbo, 2002; Tolonen and Hamalainen, 2010), in this case, on the eco-regional scale, they did not determine differences in benthic assemblages sampled through the two devices.

Those evidences observed for the structural characteristics of benthic macrofauna were more stressed from the results obtained on the community biotic indices, among which some indicating the Ecological Quality Statement. According to previous works (Quintino et al., 2011), the studied biotic indices showed a clear trend of variation from the oligohaline to the euhaline ecosystems. The pattern showed a minimum for the mesohaline ecosystems stressing the typical evidences of the model for the diversity in the estuarine environments in according to the Remane curve (Remane, 1934; Attrill and Rundle, 2002). However, the results obtained for the biotic indices were consistent with the previous on the macrofauna communities evidencing significant differences between the two samplers as already observed in other works (Alonso and Camargo, 2010).

The translation of the indices in the Ecological Quality Statement confirmed a trend already observed for corer and leaf-bag samples (Quintino et al., 2011), highlighting one class above for leaf bags in the most cases, probably reflecting the differences in the dominance of taxa for the two devices.

6. Final remarks

The results obtained in the present work have major implication for the assessment and monitoring of ecological status in TWs. They evidence that the benthic invertebrates upon which the taxonomic indices are calculated and those that contribute to the functional aspects based on the study of decomposition rates, are essentially distinct. However, the study has also implication for the typology of transitional waters in relation to which water salinity is one of the most important factor; at this regard the evidence is that on an eco-regional scale the lagoon heterogeneity (differences among lagoons) could result more important than the ecotopes (i.e., salinity groups) for determining some structural community characteristcics.

Certainly, studies of aquatic macroinvertebrate communities continue to offer opportunities for the assessment of the quality of aquatic habitats and their management requirements. However, our results evidence that more studies could be necessary to standardise appropriate methodologies to sample benthic macrofauna in transitional aquatic ecosystems in order to develop good tools or indicators for assessing the ecological quality of these ecosystems. The increasing experience of the use of these techniques by ecologists throughout Europe will inevitably lead to improvements in their application and will generate still more new approaches.

7. References

Alba-Tercedor, J., 1996. Macroinvertebrados acùaticos y calidad de las aguas de los rìos. IV Simposio del agua en Andalucìa (SIAGA). Almerìa 2, 203-231

Aller, R.C., Aller, J.Y., 1998. The effect of biogenic irrigation intensity and solute exchange on diagenetic reaction rates in marine sediments. Journal of Marine Research 56, 905-936

Alonso, A., J. A. Camargo, 2010. Comparison of two methods for estimating the abundance, diversity and habitat preference of fluvial macroinvertebrates in contrasting habitats. Limnologica 40, 23-29

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26, 32-46

Anderson, M.J, ter Braak, C.J.F., 2003. Permutation tests for multifactorial analysis of variance. Journal of Statistical Computation and Simulation 73, 85-113

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, UK, 214 pp.

Attrill, M.J., Rundle, S.D., 2002. Ecotone or ecocline: ecological boundaries in estuaries. Estuarine, Coastal and Shelf Science 55, 929-936

Barbone, E., Rosati, I., Reizopoulou, S., Basset, A., 2012. Linking classification boundaries to sources of natural variability in transitional waters: a case study of benthic macroinvertebrates. Ecological Indicators 12, 105-122

Basset A., Sabetta L., Fonnesu A., Mouillot D., Do Chi T., Viaroli P., Giordani G., Reizopoulou S., Abbiati M., Carrada G.C., 2006. Typology in Mediterranean transitional waters: new challenges and perspectives. Aquatic Conservation, Marine and Freshwater Ecosystems, 16: 441-455

Basset A., Sabetta L., Sangiorgio F., Pinna M., Abbiati M., Ponti M., Fonda Umani S., Faresi L., Simboura N., Nicolaidou A., Arvanitidis C., Moncheva S., Trajanova A., Georgescu L., Beqiraj S., Orfanidis S., Reizopoulou S., 2008. MacroBiodiversity conservation in Mediterranean and Black Sea lagoons: a traitoriented approach to benthic invertebrate guilds. Aquatic Conservation, Marine and Freshwater Ecosystems Special Issue 18, S4-S15

Basset, A., Barbone, E., A. Borja, S. Brucet, M. Pinna, X.D. Quintana, S. Reizopoulou, I. Rosati, N. Simboura, 2012. A benthic macroinvertebrate size spectra index for implementing the Water Framework Directive in coastal lagoons in Mediterranean and Black Sea ecoregions. Ecological Indicators 12, 72-83

Bocock K. L., Gilbert O.L., 1957. The disappearance of leaf litter under different woodland conditions. Plant Soil 9, 179-185

Borja A., Franco J., Pérez V., 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. Marine Pollution Bulletin 40, 1100-1114

Borja A., Muxika I., Franco, J., 2003. The application of a marine biotic index to different impact sources affecting soft-bottom benthic communities along European coasts. Marine Pollution Bulletin 46, 835-845

Cairns J., Pratt, J.R., 1993. A history of biological monitoring using benthic macroinvertebrates. Pages 10-27 in D. M. Rosenberg, V. R. Resh (eds.), Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall, New York

Castela, J., Ferreira, V., Grac¸a, M.A.S., 2008. Evaluation of stream ecological integrity using litter decomposition and benthic invertebrates. Environmental Pollution 153, 440-449

Cheal, F., Davis, J.A., Growns, J.E., Bradley, J.S., Whittles, F.H., 1993. The influence of sampling method on the classification of wetland macroinvertebrate communities. Hydrobiologia 257, 47-56

Clarke, K.R., Gorley, R.N., 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth, UK, 190 pp.

Cook, S.E., 1976. Quest for an index of community structure sensitive to water pollution. Environmental Pollution 11, 268-287

Cortes, R.M.V., Abelho, M., Rebelo, S.B., 1997. The macroinvertebrate colonization of leaf bags: is there a pattern? Limnetica 13, 71-75

Dauvin, J.C., Ruellet, T., 2007. Polychaete/amphipod ratio revisited. Marine Pollution Bulletin 55, 215-224

Elliott, M., Quintino, V., 2007. The Estuarine Quality Paradox, Environmental Homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. Marine Pollution Bulletin 54, 640-645

Emlen, J.M., 1973. Ecology: an Evolutionary Approach. Addison-Wesley: New York.

Garcìa-Criado, F., Trigal, C., 2005. Comparison of several techniques for sampling macroinvertebrates in different habitats of a North Iberian pond. Hydrobiologia 545, 103-115

Gessner, M.O., Chauvet, E., 2002. A case for using litter breakdown to assess functional stream integrity. Ecol. Appl. 12: 498-510

Gessner, M.O., Inchausti, P., Persson, L., Raffaelli, D., Giller, P.S., 2004. Biodiversity effects on ecosystem functioning: insights from aquatic systems. Oikos 104, 419-422

Guelorget, O., Perthuisot, J.P., 1983. Le Domaine Paralique. Travaux du Laboratoire de Geologie Presses de l'Ecole Normale Superieure: Paris.

Lecerf, A., Usseglio-Polatera, P., Charcosset, J.Y., Lambrigot D., Bracht B., Chauvet E., 2006. Assessment of functional integrity of eutrophic streams using litter breakdown and benthic macroinvertebrates. Arch. Hydrobiol. 165, 105-126

Legendre, L., Demers, S., 1985. Auxiliary energy, ergoclines and aquatic biological production. Le Naturaliste Canadien 112, 5-14

Levin, S.A. (ed.). 2001. Encyclopedia of Biodiversity. Academic Press: San Diego, CA.

Magni, P., Tagliapietra, D., Lardicci, C., Balthis, L., Castelli, A., Como, S., Frangipane, G., Giordani, G., Hyland, J., Maltagliati, F., Pessa, G., Rismondo, A., Tataranni, M., Tomassetti, P., Viaroli, P., 2009. Animal-sediment relationships: Evaluating the ‘Pearson–Rosenberg paradigm’ in Mediterranean coastal lagoons. Marine Pollution Bulletin 58, 478-486

Mckie, B.G., Malmqvist, B., 2009. Assessing ecosystem functioning in streams affected by forest management: increased leaf decomposition occurs without changes to the composition of benthic assemblages. Freshwater Biology 54, 2086-2100

Mistri, M, Munari, C., 2008. BITS: A SMART indicator for soft-bottom, non-tidal lagoons. Marine Pollution Bulletin 56, 580-606

Muxika, I., Borja, A., Bald, J., 2007. Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. Marine Pollution Bulletin 55, 16-29

Muzzafar, S. B., Colbo, M. H., 2002. The effects of sampling technique on the ecological characterization of shallow, benthic macroinvertebrate communities in two Newfoundland ponds. Hydrobiologia 477, 31-39

Nelson, S.M., 2000. Leaf pack breakdown and macroinvertebrate colonization: bioassessment tools for a high-altitude regulated system? Environmental Pollution 110, 321-329

Pearson, T.H., Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology Annual Review 16, 229-311

Pontasch, K., Cairns Jr., J., 1991. Multispecies toxicity tests using indigenous organisms: predicting the effects of complex effluents in streams. Arch. Environ. Contam. Toxicol. 20, 103-112

Quintino V., Sangiorgio F., Mamede R., Ricardo F., Sampaio, L., Martins, R., Freitas, R., Rodrigues A. M., Basset A., 2011. The leaf-bag and the sediment sample: Two sides of the same ecological quality story? Estuarine, Coastal and Shelf Science 95, 326-337

Remane, A., 1934. Die Brackwasserfauna. Zoologischer Anzeiger 7 (Suppl.), 34-74

Rosenberg, D.M., Resh, V.H., 1996. Use of aquatic insects in biomonitoring. In: Merritt, R.W., Cummins, K.W. (Eds.), An introduction to the Aquatic Insects of North America. Kendall/Hunt, Dubuque, IA, USA, pp. 87-96

Rosenberg, R., Blomqvist, M., Nilsson, H.C., Cederwall, H., Dimming, A., 2004. Marine quality assessment by use of benthic species abundance distribution: a proposed new protocol within the European Union Water Framework Directive. Marine Pollution Bulletin 49, 728-739

Sangiorgio F., Basset A., Pinna M., Sabetta L., Abbiati M., Ponti M., Minocci M., Orfanidis S., Arvanitidis C., Nicolaidou A., Moncheva S., Trayanova A., Georgescu L., Dragan S., Beqiraj S., Koutsoubas D., Evagelopoulos A., 2008. Environmental factors affecting *Phragmites australis* litter decomposition in Mediterranean and Black Sea transitional waters. Aquatic Conservation: Marine and Freshwater Ecosystems 18, S16-S26

Sangiorgio, F., Glazier, D.S., Mancinelli, G., Basset, A., 2010. Leaf litter decomposition in five mid-Appalachian springs (USA): the influence of habitat size. Hydrobiologia 654, 227-236

Schwoerbel, J., 1999. Einfuhrung in die Limnologie. Gustav Fischer Verlag, Stuttgart, Germany, p. 465

Shanks, R.E., Olson, J.S., 1961. First year breakdown of leaf litter in Southern Appalachian forest. Ecology 134, 194-195

Simboura, N., Zenetos, A., 2002. Benthic indicators to use in ecological quality classification of Mediterranean soft bottom marine ecosystems, including a new biotic index. Mediterranean Marine Science 3, 77-111

Somerfield, P.J., Clarke, K.R., 1997. A comparison of some methods commonly used for the collection of sublittoral sediments and their associated fauna. Marine Environmental Research 43, 145-156

Stein, H., Springer, M., Kohlman, B., 2008. Comparison of two sampling methods for biomonitoring using aquatic macroinvertebrates in the Dos Novillos River, Costa Rica. Ecological Engineering 34, 267-275

Tolonen, K. T, Hamalainen, H., Holopainen, I. J., Karjalainen, J., 2001. Influence of habitat type and environmental variables on littoral macroinvertebrate communities in a large lake system. Arch. Hydrobiol. 152, 39-67

Tolonen, K. T., Hamalainen, H., 2010. Comparison of sampling methods and habitat types for detecting impacts on lake littoral macroinvertebrate assemblages along a gradient of human disturbance. Fundamental Applied Limnology, Arch. Hydrobiol. 176, 43-59

Weisberg, S.B., Ranasingue, J.A., Dauer, D.M., Schaffner, L.C., Diaz, R.J., Frithsen, J.B., 1997. An estuarine benthic index of biotic integrity (B-IBI) for Chesapeake Bay. Estuaries 20, 149-158

Annex 1. Taxa of aquatic macroinvertebrates only found either with the box-corer or the leaf-bag method in the studied lagoons. For each taxon the functional feeding group ([FFG] D-SH detritivorous shredders, D-SC detritivorous scrapers, D-C detritivorous filtering or gathering collectors , P predators, OTH others) is reported. (x = presence of taxa).

Taxon		Method		FFG
Phylum		Box-corer	Leaf bag	
Cnidaria	Actiniaria [n.d.]	x	x	P
Platyhelminthes	Planariidae spp.	x	x	P
	Platyhelmintes [n.d.]		x	P
	Tricladida [n.d.]		x	P
	Turbellaria [n.d.]		x	P
Echinodermata	Ophiuridae spp.	x	x	P
	Trachythyone elongata	x		D-C
Nematoda	Enoplida [n.d.]	x		P
	Nematoda [n.d.]	x	x	OTH
Nematomorpha	Nematomorpha [n.d.]	x	x	P
Nemertea	Nemertea [n.d.]		x	P
Mollusca	*Abra prismatica*	x		D-C
	Abra segmentum	x	x	D-C
	Acanthocardia echinata	x		D-C
	Ancylidae spp.		x	D-SC
	Anisus sp.		x	D-SC
	Bithynia sp.		x	D-SC
	Bithynia tentaculata	x	x	D-SC
	Bittium reticulatum	x	x	D-SC
	Bivalvia [n.d.]		x	D-C
	Cardiidae spp.		x	D-C
	Cerastoderma glaucum	x	x	D-C
	Cerithium sp.	x		D-SC
	Cyclope neritea	x	x	D-SC
	Dosinia lupinus	x	x	D-C
	Dreissena sp.	x	x	D-C
	Eulimella ventricosa		x	D-SC
	Flabellina sp.		x	P
	Gastrana fragilis	x		D-C
	Gastropoda [n.d.]		x	OTH
	Gibbula adansonii		x	D-SC
	Haminoea navicula	x		D-SC
	Haminoea sp.	x	x	D-SC
	Hydrobia acuta		x	D-SC
	Hydrobiidae spp.		x	D-SC
	Lepetidae spp.		x	D-SC
	Littorina neritoides		x	D-SC
	Loripes lacteus	x	x	D-C

Annex 1. Continued

Taxon		Method		FFG
Phylum		Box-corer	Leaf bag	
Mollusca	*Mytilaster* sp.	x	x	D-C
	Mytilus galloprovincialis		x	D-C
	Nassarius reticulatus	x		D-SC
	Nassarius sp.	x	x	D-SC
	Naticidae spp.	x		P
	Paludinella littorina		x	D-SC
	Paphia aurea	x		D-C
	Physidae spp.		x	D-SC
	Planorbarius corneus	x	x	D-SC
	Planorbarius sp.	x	x	D-SC
	Planorbis sp.	x	x	D-SC
	Potamidae spp.		x	D-SC
	Pusillina sp.		x	D-SC
	Rissoidae spp.		x	D-SC
	Scrobicularia cottardi		x	D-C
	Scrobicularia plana	x	x	D-C
	Tapes sp.	x	x	D-C
	Tellina sp.	x		D-C
	Theodoxus fluviatilis	x	x	D-SC
	Valvatidae spp.	x	x	D-SC
	Venerupis sp.		x	D-C
	Ventrosia ventrosa	x	x	D-SC
Annelida	*Batracobdella* sp.	x	x	P
	Capitella capitata	x		D-C
	Cirratulidae spp.		x	D-C
	Cirratulus sp.	x		D-C
	Criodrilidae spp.	x		D-C
	Enchytraeidae spp.	x		D-C
	Erpobdella sp.	x	x	P
	Ficopomatus enigmaticus	x	x	D-C
	Glycera sp.	x		P
	Glossiphonia sp.		x	P
	Haplotaxidae spp.	x	x	D-C
	Hediste diversicolor	x		P
	Helobdella sp.		x	P
	Heteromastus filiformis	x		D-C
	Lumbricidae spp.	x	x	D-C
	Lumbrineridae spp.	x	x	D-C
	Malacoceros fuliginosus	x		D-C
	Naididae spp.		x	D-C
	Naidinae spp.	x		D-C
	Naineris laevigata	x	x	D-C

Annex 1. Continued

Taxon		Method		FFG
Phylum		Box-corer	Leaf bag	
Annelida	Nephtyidae spp.	x	x	P
	Nephtys hombergii	x		P
	Nereididae spp.	x	x	P
	Nereis diversicolor		x	P
	Nereis sp.	x		P
	Notomastus sp.	x		D-C
	Oligochaeta [n.d.]	x	x	D-C
	Opheliidae spp.	x		P
	Paraonidae spp.	x		D-C
	Perinereis cultrifera		x	D-C
	Phyllodocidae spp.	x	x	P
	Piscicola sp.	x	x	P
	Polychaeta [n.d.]	x	x	OTH
	Polydora ciliata	x		D-C
	Prionospio cirrifera	x		D-C
	Sabellidae spp.	x	x	D-C
	Spionidae spp.	x	x	D-C
	Sternaspis scutata	x		D-C
	Streblospio shrubsolii	x		D-C
	Streptosyllis sp.		x	D-C
	Syllidae spp.	x	x	P
	Tubificidae spp.	x	x	D-C
Arthropoda	*Acentria* sp.	x		D-SH
	Aeshna mixta [larval stage]	x	x	P
	Ampelisca diadema	x		D-C
	Amphipoda [n.d.]		x	OTH
	Artemia salina	x	x	D-C
	Asellidae spp.	x	x	D-SH
	Asellus aquaticus	x		D-SH
	Asellus sp.	x	x	D-SH
	Balanus improvisus		x	D-C
	Brachyura [n.d.]		x	P
	Caenis sp.	x	x	D-SH
	Caprella acanthifera	x		P
	Carcinus maenas		x	P
	Ceratopogonidae spp.	x	x	P
	Chironomidae spp.	x	x	D-C
	Chironomus plumosus	x	x	D-C
	Chironomus salinarius	x	x	D-C
	Chironomus sp.	x	x	D-C
	Coleoptera [n.d.]	x		OTH
	Corophium sp.	x	x	D-C
	Crangon crangon	x		P
	Cumacea [n.d.]	x	x	D-C
	Cyathura carinata		x	P
	Dexamine sp.	x	x	D-SH

Annex 1. Continued

Taxon		Method		FFG
Phylum		Box-corer	Leaf bag	
Arthropoda	*Dexamine spinosa*	x		D-SH
	Diamesinae spp.	x	x	D-SH
	Diptera [n.d.]	x	x	OTH
	Echinogammarus sp.		x	D-SH
	Ecnomidae spp.	x	x	D-C
	Elasmopus sp.	x		D-SH
	Ephemerella sp.	x		D-C
	Ephydridae spp.		x	P
	Gammarella fucicola		x	D-SH
	Gammaridae spp.	x	x	D-SH
	Gammarus aequicauda	x		D-SH
	Gammarus insensibilis	x	x	D-SH
	Gammarus sp.	x	x	D-SH
	Harpinia crenulata	x		D-C
	Hemiptera [n.d.]			
	Idotea balthica	x	x	D-SH
	Idotea sp.		x	D-SH
	Iphinoe serrata	x		D-C
	Isopoda [n.d.]	x	x	OTH
	Jaera hopeana		x	D-SH
	Lekanesphaera hookeri	x	x	D-SH
	Lekanesphaera monodi	x	x	D-SH
	Lepidoptera [n.d.]	x		OTH
	Leptoceridae [n.d.]	x		D-C
	Lestes sp.	x	x	P
	Leucothoe sp.		x	D-SH
	Limnoria lignorum	x		D-SH
	Liocarcinus arcuatus	x		P
	Melita palmata		x	D-SH
	Microdeutopus anomalus		x	D-SH
	Microdeutopus gryllotalpa	x	x	D-SH
	Microdeutopus sp.	x	x	D-SH
	Mysidae spp.	x	x	P
	Nymphula nymphaeata	x		D-SH
	Odonata [n.d.]		x	P
	Perioculodes aequimanus	x		D-C
	Porcellanidae spp.		x	D-C
	Portunidae spp.		x	P
	Psychomyiidae spp.	x		D-SH
	Sphaeromatidae spp.		x	D-SH
	Stratiomyidae spp.	x	x	D-SC
	Tabanidae spp.	x	x	P
	Tanaidacea [n.d.]	x		D-SH
	Tanais dulongii		x	D-SH
	Tanypodinae spp.	x	x	P
	Tipulidae spp.	x		D-SH
	Trichoptera [n.d.]	x		D-SH